

PKM2

PDB:3GR4

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:GI:33286417

Entry Clone Source:MGC

SGC Clone Accession:

Tag:N-terminal histag with thrombin cleavage site: mgsshhhhhssglvprgs

Host:BL21 DE3

Construct

Prelude:

Sequence:

mgsshhhhhssglvprgsMSKPHSEAGTAFIQTLHAAMADTFLEHMCRLDIDSPITARNTGIICTIGPASRSVETLKEMIKSG
MNVARLNFSGHGTHEYHAETIKNVRTATESFASDPILYRPVAVALDTKGPEIRTGLIKSGTAEVELKKGATLKITLDNAYMEKCDEN
ILWLDYKNICKVVEVGSKIYVDDGLISLQVKQKGAFLVTEVENGGSLGSKKGVNLPAAVDLPVSEKDIQDLKFGVEQDVMVFA
SFIRKASDVHEVRKVLGEKGKNIKIISKIENHEGVRRFDEILEASDGIMVARGDLGIEIPA EKVF LAQMMIGRCNRAGKPVICATQ
MLESMIKKPRPTRAEGSDVANAVLDGADCIMLSGETAKGDYPLEAVRMQH LIAREAEAAIYHLQLFEELRRLAPITSDPTEATAVGA
VEASFKCCSGAIIVLTKSGRSAHQVARYRPRAPIIAVTRNPQTARQAHL YRGIFPVLCKDPVQEAWAEDVDLRVNFAMNVGKARGFF
KKGDVVIVLTGWRPGSGFTNTMRVVPVP

Vector:p28a-LIC

Growth

Medium:

Antibiotics:

Procedure:We prepared the seeds by inoculating freshly transforming E. coli cells (BL21 DE3) into 80 mL of Luria-Bertani medium. After overnight, all of the seeds were inoculated into 1.8 L of Terrific Broth medium in the presence of 50 µg/mL of kanamycin at 37°C and grown to an OD600 of 4.0. Cells were then induced by isopropyl-1-thio-D-galactopyranoside at the final concentration of 1.5 mM and grown overnight at 20°C in a LEX bubbling system.

Purification

Procedure

The supernatant was passed through DE52 (Whatman) column equilibrated with the binding buffer and then loaded onto 3 mL Ni-NTA column (Qiagen) equilibrated with the same binding buffer at 4 °C. The Ni-NTA column was washed with 150 mL of the wash buffer and the protein was eluted with 15 mL of the elution buffer. The eluate was dialyzed overnight against a buffer

containing 10 mM HEPES pH 7.5, 150mM KCl, 2mM TCEP, 5% glycerol, and 5mM MgCl₂. The protein concentration was estimated based on the extinction coefficient of the protein, 29190 at 280 nm. Five molar equivalents of ADP, 5 mM TCEP and 5 mM MgCl₂ were added to the purified protein before concentration. The protein was concentrated using an Amicon Ultra centrifugal filter to the final volume of 1 mL and the concentration of 30 mg/mL. About 55 mg of protein was obtained from 1.8 L of cell culture.

Extraction

Procedure

Cultures were centrifuged and the cell pellets were suspended in 100 mL of the binding buffer with a protease inhibitor cocktail (0.1 mM M benzamidine-HCl and 0.1 mM phenylmethyl sulfonyl fluoride) and flash frozen. The thawed cell pellet was lysed by a combination of 0.5% CHAPS (Sigma) and sonication. The lysate was centrifuged at 15000 rpm for 30 min and the supernatant was used for subsequent steps of purification.

Concentration:30 mg/mL

Ligand

MBB01-45**MassSpec:**

Crystallization:The protein was mixed with 5-10 fold molar excess of the activator and incubated overnight at room temperature. Crystals were obtained by the sitting drop vapor diffusion method, 1ul of the protein was mixed with 1 ul of unbuffered reservoir solution consisting of 20% PEG3350, 0.2M di-NH₄-tartrate. Plate-like crystals were grown within three days. For data collection a single crystal was separated from the cluster and cryoprotected in a 50:50 mixture of Paratone-N and mineral oil before flash cooling in liquid nitrogen.

NMR Spectroscopy:

Data Collection:

Data Processing: